REFERENCES

[1] J. B. THOMAS, M. BUSTRAAN, AND C. H. PARIS, *Biochim. Biophys. Acta*, 8 (1952) 90.
[2] E. MONNÉ, *Advances in Enzymol.*, 8 (1948) 1.
[3] P. RONDONI, *Ergebn. Enzymforsch.*, 10 (1949) 264.
[4] L. MARTON AND L. I. SCHIFF, *J. Appl. Phys.*, 12 (1941) 759.

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SUCROSE SYNTHESIS IN HIGHER PLANTS AND HIGH ENERGY PHOSPHATE

by

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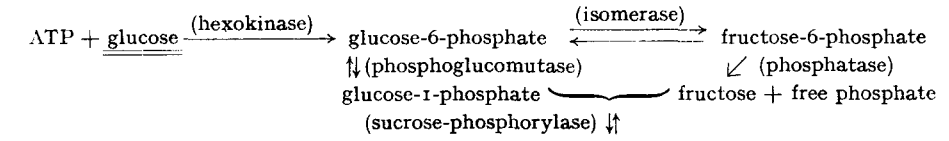
Literature data[1,2,5,6] as well as experiments carried out on germinating rice by the present authors, clearly show that aerobic respiration is necessary for the synthesis of sucrose in higher plants. The following experiments offer an explanation for this phenomenon. Potatoes (variety Doré) were kept in the dark at 30° C for a couple of weeks and slices were cut from the small tubers ("submarines") formed by them. These slices were exposed to the action of 20% sugar solutions at pH 7 for 1 hr; after this, they were superficially dried and kept in a moist desiccator for 18–20 hr (compare 6). Both glucose and fructose gave a great increase in sucrose, *viz.*, up to 177% in addition to the amount already present. 20% mannitol gave a 72% decrease, presumably owing to respiration; this means that the plasmolysis caused by the hexose solutions probably is not, or not solely, responsible for their effect. Inorganic phosphate strongly inhibited sucrose formation, which *may* mean that, in the final step of sucrose synthesis, a liberation of inorganic phosphate takes place. Vacuum infiltration of the slices with the solutions used[4] or replacement of air by nitrogen prevented sucrose synthesis, *unless ATP was added beforehand.* It is a well-established fact that much more of this substance is produced in aerobic than in anaerobic respiration. AMP or ADP could not replace ATP, so that the effect of the latter substance cannot be due to the presence of the adenosine ring system alone. Magnesium ions had an activating effect upon sucrose synthesis, certain concentrations of fluoride an inhibiting one. Glyceraldehyde and its biological precursor, L-sorbose-1-phosphate (both known to be hexokinase poisons!) inhibited synthesis. As the present authors have been able to obtain a fairly active hexokinase concentrate from potato press-juice, and as the enzyme has since then also been reported in the same material by KOTELNIKOVA[3], it is reasonable to assume that the function of ATP in sucrose synthesis is to make possible the action of hexokinase. Experiments with glucose-6-phosphate have shown that this must be the only function of ATP here, for glucose-6-phosphate under anaerobic circumstances gives only an insignificant decrease in sucrose, and sometimes even a small increase. Other enzymes demonstrated in potato press-juice by the present authors are: phosphoglucomutase, phosphohexoisomerase and, surprisingly, a phosphatase which, at pH 7, exerts its action mainly on fructose-6-phosphate. It is the presence of this latter enzyme with its power to produce free phosphate which may account for the lack of success encountered by the present authors when they tried sucrose synthesis from fructose-6-phosphate or from Robison-ester. It is clear that, if only sucrose phosphorylase could be demonstrated in potatoes, sucrose synthesis there might be pictured as follows:



The thin evidence for the presence of sucrose phosphorylase that could be obtained is mainly based on experiments in which potato press-juice was allowed to act, at pH 7, on a mixture of sucrose and inorganic phosphate, in the presence of a small amount of notatin added to eliminate selectively the interfering substance glucose. In most cases, a decrease in inorganic phosphate and a roughly corresponding increase in 7-min-phosphate was observed. This 7-min-phosphate could not be identified with certainty yet, but at least part of it seems to be glucose-1-phosphate. Sucrose could not be replaced by other sugars, with the exception of maltose, a substance known to be phosphorylated

by, *e.g.*, pea extracts (J. TURNER, oral communication). It was shown that the sucrose effect could not be ascribed to an activation of dextrin phosphorylation. That it was based on enzyme action is likely from the fact that it showed an optimum at pH 7 and 35° C. The only way in which a (slight) concentration of activity could be achieved was to keep the press juice in a cold desiccator overnight. Unfortunately, as the autumn season proceeded, the results obtained earlier with untreated juices and concentrates could not be reproduced. Therefore, it is quite possible that the enzyme (if present at all) shows rather strong fluctuations in activity or stability during the course of the year. Experiments to investigate this possibility and to compare various races of potatoes are in progress.

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REFERENCES

1 P. BOYSEN-JENSEN, *Biochem. Z.*, 40 (1912) 420.
2 C. E. HARTT, *Hawaiian Planters' Record*, 47, nos. 2, 3 and 4 (1943); *ibid.*, 48, no. 1 (1944).
3 A. V. KOTELNIKOVA, *Doklady Akad. Nauk S.S.S.R.*, 78 (1951) 737; *C. A.*, 45 (1951) 10311 c, d.
4 G. KROTKOV, *Science*, 105 (1947) 318.
5 O. A. LEONARD, *Am. J. Botany*, 25 (1938) 78; *ibid.*, 26 (1939) 475.
6 J. M. NELSON AND R. AUCHINCLOSS, *J. Am. Chem. Soc.*, 55 (1933) 3769.

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THE THIAMINE PYROPHOSPHATE CONTENT OF CENTRIFUGALLY-PREPARED FRACTIONS OF RAT LIVERHOMOGENATE. THE INFLUENCE OF A THIAMINE-DEFICIENT DIET*

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By various investigators[1,2,3] it has been fairly well established that the enzymes of the tricarboxylic acid cycle are essentially associated with the mitochondrial units of the cell. It is known that enzymes containing thiamine pyrophosphate are involved in the breakdown of pyruvic- and α-ketoglutaric acids[4], which are both oxidized by means of the tricarboxylic acid cycle.

Furthermore enzymes requiring thiamine pyrophosphate, which catalyze anaerobic reactions, were found to be associated with particles which can be sedimented quantitatively after 30 minutes at 15.000 r.p.m. in the high speed head of the International centrifuge[5].

Therefore it seemed of interest to determine whether thiamine pyrophosphate is bound entirely to the mitochondria. For isolating the mitochondria as well as the other cell components, the procedure of HOGEBOOM, SCHNEIDER, AND PALLADE[6] was followed with the modification that the washing of the microsomes was omitted.

The thiamine pyrophosphate contents of the isolated fractions were determined by the manometric method of WESTENBRINK AND STEYN-PARVÉ. In each experiment 1 g of liver pulp of an adult, male, Wistar rat was fractionated.

Table I shows that the amounts of thiamine pyrophosphate found in the microsomes are insignificant. They may be due to material originating from the soluble fraction because the preparations were not washed.

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